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Isolation and Identification of Dermatophytes in a Tertiary Care Hospital

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A B S T R A C T

There is an increased prevalence of dermatophytes over the past few decades. This study was done to isolate and identify dermatophytes in patients attending dermatology outpatient department in a tertiary care hospital. A total of 100 skin, hair and nail samples from clinically suspected cases of dermatophytosis of all age groups and of both sexes were collected and processed by KOH and culture on Sabouraud dextrose agar and Dermatophyte test media. Out of 100 clinically suspected cases of Dermatophytosis, commonly affected age group was 21-30 yrs. (M: F = 1.43:1). Culture positive cases were 40%. Sensitivity and Specificity for KOH and culture of all samples were 90% and 75% respectively. Distribution of clinical lesions based on site were *Tinea corporis* (31%), *T. unguium* (26%), *T. cruris* (25%), *T. facei* (6%), *T. capitis* (4%), *T. pedis* (4%), *T. barbae* (2%), *T. manuum* (2%). Frequency of various species of dermatophytes isolated by culture were *Trichophyton tonsurans* (42.5%), *T. mentagrophytes* (20%) *T. verrucosum* (20%), *T. rubrum* (7.5%), *Epidermophyton floccosum* (5 %), *T. violaceum* (2.5 %) and *Microsporum gypseum* (2.5%). To conclude, in this study, most common clinical type of dermatophytosis was *Tinea corporis*. Most common dermatophyte isolated from skin scrapings was *T. tonsurans* followed by *T. mentagrophytes*. Dermatophytes isolated from nail samples were *T. verrucosum* and *T. tonsurans*. Dermatophyte isolated from hair was *T. violaceum*.

Introduction

The dermatophytes are a group of closely related fungi that have the capacity to invade the keratinized tissues of skin, hair and nails and cause an infection, dermatophytosis, commonly referred to as ringworm or tinea. The dermatophytes are included in three fungal genera *viz.*, 1. *Epidermophyton*, 2. *Microsporum* and 3. *Trichophyton.*

These fungi colonize in the keratin tissues and are frequently restricted to the nonliving cornified layer of the epidermis. Dermatophytes are also associated with secondary bacterial infections leading to systemic skin infections. According to WHO, the prevalence rate of superficial mycotic infection worldwide has been found to be 20-25%. Dermatophytic infections are commonly encountered in more than 50% of patients attending dermatology outpatient departments in South India.

The tinea infections are prevalent globally but they are common in tropics and in geographical areas with higher humidity, over population and poor hygienic living

conditions. The organisms are transmitted by either direct contact or indirect contact (fomites).

An increasing frequency of dermatophytosis has been observed during last two decades especially in immunocompromised patients such as AIDS, diabetes mellitus, cancer and organ transplantation patients, etc.

Dermatophytoses generally respond well to topical antifungal therapy, although systemic therapy would be required for extensive infections or for infections affecting the nails or scalp.

The present study was conducted to isolate and identify dermatophytes from skin, hair and nail samples of clinically suspected cases of dermatophytosis by KOH and culture on SDA and DTM media.

Materials and Methods

The present study has been carried out on 100 clinically suspected cases of dermatophytosis in all age groups and of both sexes, attending the outpatient department of Dermatology, Venereology and Leprosy at Gandhi Hospital, Musheerabad from April 2015 to August 2016.

A prospective cross sectional study was planned after obtaining permission from Institutional ethics committee.

Inclusion criteria

Clinically suspected cases of dermatophytoses of all age groups attending dermatology OPD of Gandhi hospital

Exclusion criteria

Cases of dermatophytosis those are on treatment with antifungal drugs.

Cases of dermatophytosis with secondary bacterial infection.

All relevant details like age, sex, occupation, duration of presenting complaints and site of involvement were taken in a structured proforma.

Detailed history of involvement of any other family member, history of diabetes mellitus or any immunocompromised state

Methods

Specimen collection

Skin, hair and nail samples were collected from clinically suspected cases of dermatophytosis under aseptic conditions using 70% alcohol into a sterile black paper.

Skin scrapings

The scrapings were collected from the periphery of the lesions with a sterile scalpel blade.

Hair pluckings

Dull broken hairs from the margin of the lesion were plucked using sterile tweezers or by scraping the scalp with a blunt scalpel.

Nail clippings

Deeper fragments which included crusty deposits from the junction of nail were collected with the help of sterile scissors or nail clippers.

Microscopical examination of specimens

KOH wet mount

The specimens collected were subjected to KOH wet mount preparation. 10% KOH used

for skin and hair samples and kept for 10-15 minutes and 40% KOH used for nail samples, kept overnight. A small amount of sample is taken and added to the drop of KOH placed on a glass slide and a cover slip is placed on it.

On microscopy, branching hyaline mycelia which frequently show arthrospores production were seen (Table 4 and 5).

Culture

Irrespective of demonstration of fungal elements on microscopy, the specimen was inoculated onto Sabouraud's dextrose agar with 0.05% Chloramphenicol and 0.5% Cycloheximide and incubated at 28°C for up to four weeks, and was observed periodically for growth. If no growth was found after four weeks, it was taken as negative for fungal growth.

Macroscopic examination of culture

The growth on Sabouraud's dextrose agar was examined to study the colony morphology based on following characteristics.

On obverse for colour and consistency and on reverse for the presence or absence of pigment, whether diffusing or not.

Tease mount

By lactophenol cotton blue

A small portion of a colony was picked and suspended in two drops of lactophenol cotton blue placed on a clean slide.

The mycelial mat was teased apart with dissecting needles, covered with cover-slip and observed under microscope for presence of aseptate slender hyphae, macro and microconidia and their arrangement.

Dermatophyte Test Media (DTM)

This medium was used to confirm whether the fungus grown was dermatophyte. All isolated dermatophytes were inoculated onto DTM and incubated at 28°C for 7 days and observed for color change. Color change of the medium from yellow to red indicated growth of dermatophytes. All species of dermatophytes showed this color change.

Dermatophyte species were further confirmed based on urease test

Urease test

This test is to differentiate between *T. mentagrophytes* and *T. rubrum.* Christensen's urea agar slant was inoculated with the test fungus. *T. mentagrophytes* hydrolyses urea usually within seven days and colour of the medium changes to pink. T. rubrum isolates were negative for urease test.

Results and Discussion

Gender wise distribution of cases

Out of 100 clinically suspected cases, males were more commonly effected (59 %) than females (41 %). Highest incidence of cases was between 21 and 30 years age group with 37% followed by 31 and 40 years with 18 %. Least incidence was observed in the age group above 60 years with 5 % cases. Males were more prominent in age group 21- 30 yrs whereas females were more affected in age group 31-40 yrs.

The socioeconomic status of patients was estimated based on income, education, and occupation. Most patients (64%) were from low socioeconomic status. Most common clinical type observed was Tinea corporis (31%) followed by Tinea unguium (26%) and Tinea cruris (25%).

Clinical sample distribution in study population

Skin- 69, Nail -27, Hair -4 out of 100 samples

Out of 100 clinical cases of dermatophytosis, skin scrapings were the predominant clinical sample, followed by nail clippings and hair pluckings.

Out of 100 samples, 51 were positive by KOH mount. Out of 51 direct microscopy positive 44 (63.76%) were skin, 6 (22.22%) were nail and 1 (25%) was a hair sample. Out of 100 samples, 40 were positive for culture. Out of the 40 samples, 37 (53.62%) were skin, 2 (7.4%) were nail and 1 (25%) was hair. Out of 40 isolated dermatophytes, majority were *Trichophyton tonsurans* (42.5%), followed by *Trichophyton mentagrophytes* (20%) and *Trichophyton verrucosum* (20%), *Trichophyton rubrum* (7.5%), *Epidermophyton floccosum* (5 %), *Trichophyton violaceum* (2.5 %) and *Microsporum gypseum* (2.5%).

Most common dermatophyte isolated from skin scrapings was *Trichophyton tonsurans* (16) followed by *Trichophyton mentagrophytes* (8). Dermatophytes isolated from nail samples were *Trichophyton verrucosum* (1) and *T. tonsurans* (1). Dermatophyte isolated from hair was T. violaceum (1)

Dermatophytes are molds affecting keratinized tissue causing superficial mycoses in humans and animals commonly known as ringworm infection. In the present study, 100 clinically suspected cases of dermatophytosis were studied at Gandhi Hospital, Musheerabad during a period of one and half years. The study was undertaken to isolate and identify dermatophytes. Out of 100 clinically suspected cases of dermatophytoses in the present study, males were more commonly effected (59 %) than females (41 %). This correlates with the studies Poluri LV *et al.,* (2015), Noronha *et al.,* (2016). Male predominance could be due to increased outdoor physical activity and increased opportunity for exposure to infection than females.

The predominant age group in this study was 21- 30 yrs which correlated with other studies done by Poluri *et al.,* (2015) and Noronha *et al.,* (2016). The higher incidence of dermatophytes in young age may be due to increased physical activity, increased opportunity for exposure and hormonal pattern.

In this study dermatophytosis was more common in low socioeconomic group (64%) followed by middle income group (34%) and high income group (2%). This is in correlation with the study by Poluri *et al.,* (2015) in which low income group comprised 67%. This may be due to poor hygienic conditions, large family size, close contact, sharing unwashed clothes, combs and towels and also due to malnutrition.

An infected family member is also an important source of infection in superficial mycoses. In our study, family history of superficial fungal infections was seen in 23 % cases similar to a study by Monika *et al.,* (2016) who reported a family history in 33% cases. Insanitary conditions and sharing of fomites within the family contributes to the spread of infection. Out of 100 cases, 10 cases presented with predisposing conditions. Diabetes mellitus was seen in 9 cases (9%) out of which 6 were culture positive. There was a single case of onychomycosis with Rheumatoid arthritis on immunosuppressive drugs since 2yrs.In a study by Poluri *et al.,* (2015) systemic predisposing factors like diabetes, anemia, atopy, and HIV were observed.

Table.1 Age and gender wise distribution of study population

Age Group (in years)	Males	Females	Total	Percentage
upto 20	8	9	17	17%
21 – 30	26	11	37	37 %
31 – 40	6	12	18	18 %
41 – 50	11	5	16	16 %
51 – 60	5	2	7	7 %
> 60	3	2	5	5%
TOTAL	**59**	**41**	**100**	**100**

Table.2 Socio economic status

Class	No. of cases	Percentage
Upper	**2**	**2%**
Middle	**34**	**34%**
Low	**64**	**64%**

Table.3 Distribution of clinical lesions in study population based on site involved

Clinical Diagnosis	Males	Females	Total no. of cases	Percentage
Tinea capitis	2	2	4	4%
Tinea facei	5	1	6	6%
Tinea barbae	2	-	2	2%
Tinea corporis	15	16	31	31%
Tinea manuum	2	0	2	2%
Tinea unguium	15	11	26	26%
Tinea cruris	16	9	25	25%
Tinea pedis	2	2	4	4%

Table.4 Fungi demonstrated by direct microscopy (KOH) and culture

	No. of samples (n=100)	KOH (positive) (n=51)	Positivity %	Culture (positive)	Positivity %
Skin	69	44	63.76%	37	53.62%
Nail	27	6	22.22%	2	7.4%
Hair	4	1	25%	1	25%
Total	**100**	**51**		**40**	

Table.5 Direct microscopy (KOH) and culture results in all 100 samples

	Culture Positive	**Culture Negative**	**Total**
KOH Positive	36	15	51
KOH Negative	4	45	49
Total	40	60	100

Table.6 Sensitivity, specificity, positive predictive value and negative predictive value in skin, nail and hair samples

TYPE OF SPECIMEN	**Sensitivity (TP/ TP+FN)**	**Specificity (TN/TN+FP)**	**Positive predictive value (TP/ TP+FP)**	**Negative predictive value (TN/TN+FN)**
SKIN	91.89%	68.75%	77.27%	88%
NAIL	50%	80%	16.66%	95.23%
HAIR	100%	100%	100%	100%

TP = True positive, TN= True negative, FP= False positive, FN= False negative

Table.7 Frequency of various species of dermatophytes isolated

DERMATOPHYTE SPECIES	**NUMBER**	**PERCENTAGE**
T.mentagrophytes	8	20 %
T.rubrum	3	7.5 %
T.verrucosum	8	20%
M.gypseum	1	2.5 %
T.tonsurans	17	42.5 %
E.floccosum	2	5%
T.violaceum	1	2.5 %
TOTAL	**40**	**100**

Table.8 Dermatophytic isolates from different clinical samples of tinea

DERMATOPHYTE	**SKIN n=69**	**NAIL n=27**	**HAIR n=4**	**TOTAL n=100**
T.mentagrophytes	8(11.59%)	0(0%)	0(0%)	8
T.rubrum	3(4.34%)	0(0%)	0(0%)	3
T.verrucosum	7(10.14%)	1(3.70%)	0(0%)	8
T.tonsurans	16(23.18%)	1(3.70%)	0(0%)	17
T.violaceum	0 (0%)	0(0%)	1(25%)	1
E.floccosum	2(2.89%)	0(0%)	0(0%)	2
M.gypseum	1(1.44%)	0(0%)	0(0%)	1
TOTAL	**37**	**2**	**1**	**40**

Table.9 Dermatophytic isolates in different clinical types of tinea

Fungal Isolates	*Tinea capitis*	*Tinea facei*	*Tinea barbae*	*Tinea corporis*	*Tinea manuum*	*Tinea unguium*	*Tinea cruris*	*Tinea pedis*	Total
T.mentagrophytes	0	0	0	5	0	0	3	0	8
T.rubrum	0	0	0	2	0	0	0	1	3
T.verrucosum	0	1	0	2	0	1	4	0	8
T.tonsurans	0	0	1	11	0	1	4	0	17
T.violaceum	1	0	0	0	0	0	0	0	1
E.floccosum	0	0	0	1	0	0	1	0	2
M.gypseum	0	0	0	0	0	0	1	0	1
Total	1	1	1	21	0	2	13	1	40

FIGURE 1:Tinea facei

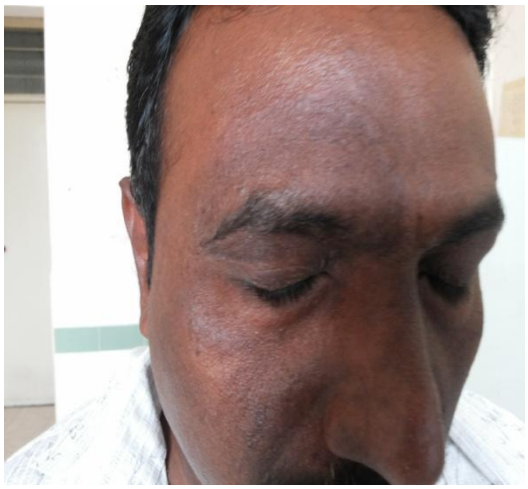


FIGURE 2:Tinea barbae

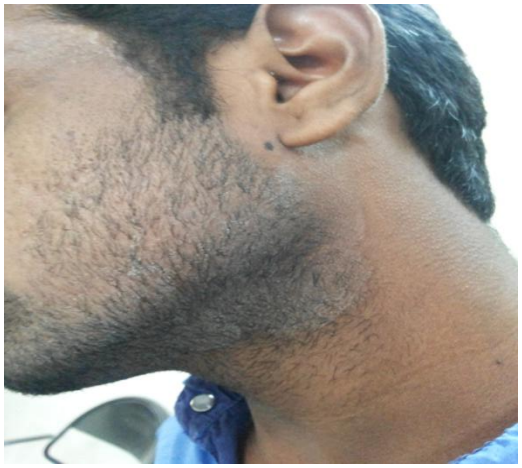


FIGURE 3: Tinea corporis

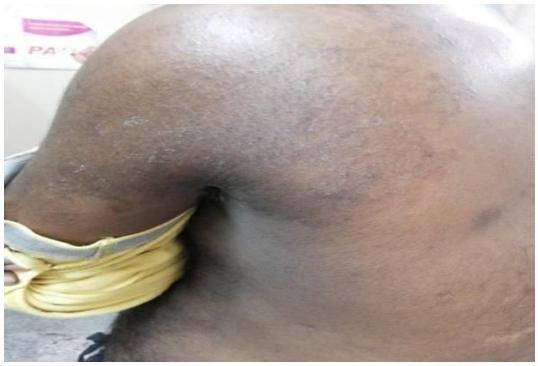


FIGURE 4:Tinea cruris

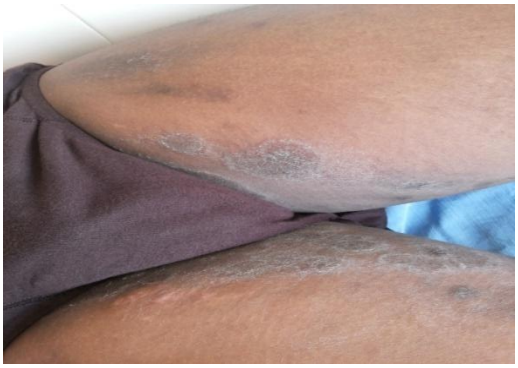


FIGURE 5: Tinea mannum

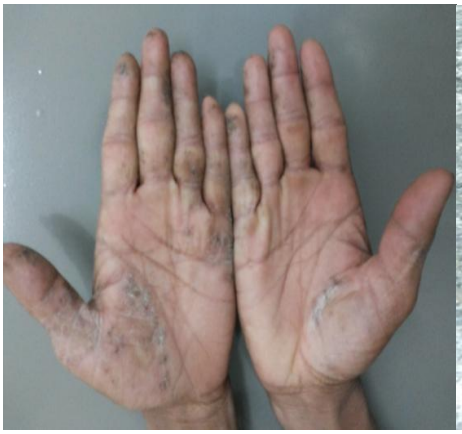


FIGURE 6 :Tinea pedis

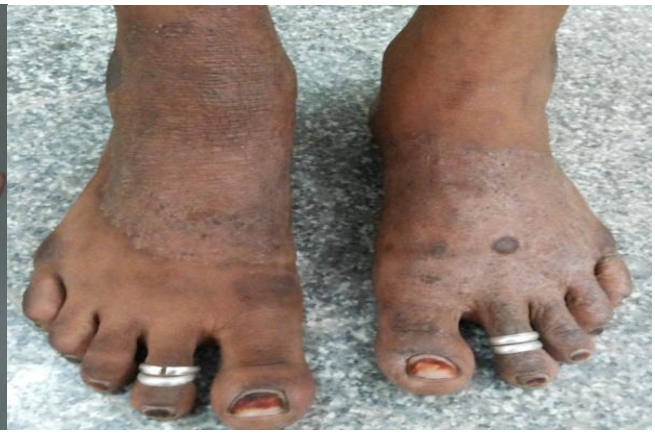


FIGURE 7:Tinea unguium

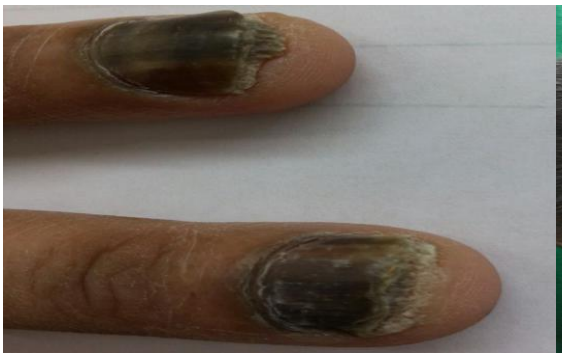


FIGURE 8:Tinea capitis

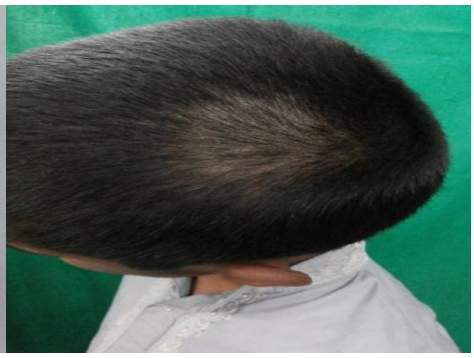
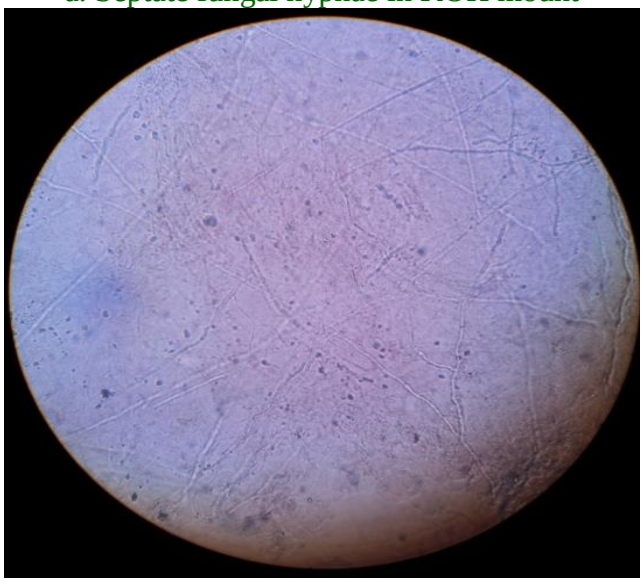


Fig.9 Direct Microscopy

a. Septate fungal hyphae in KOH mount



b. Spores in endothrix type of Tinea capitis

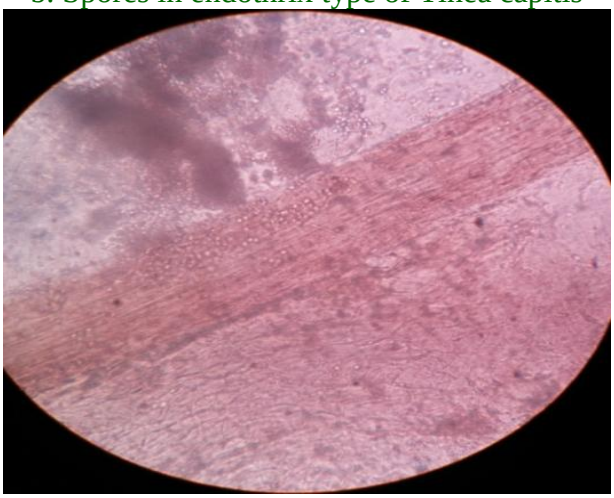


Fig.10 Lactophenol cotton blue

Fig.11 *Trichophyton mentagrophytes*

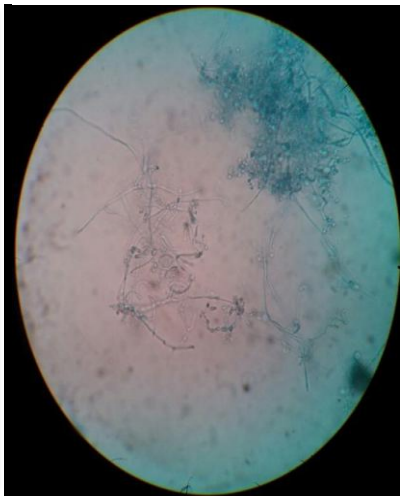


Fig.12 *Trichophyton rubrum*

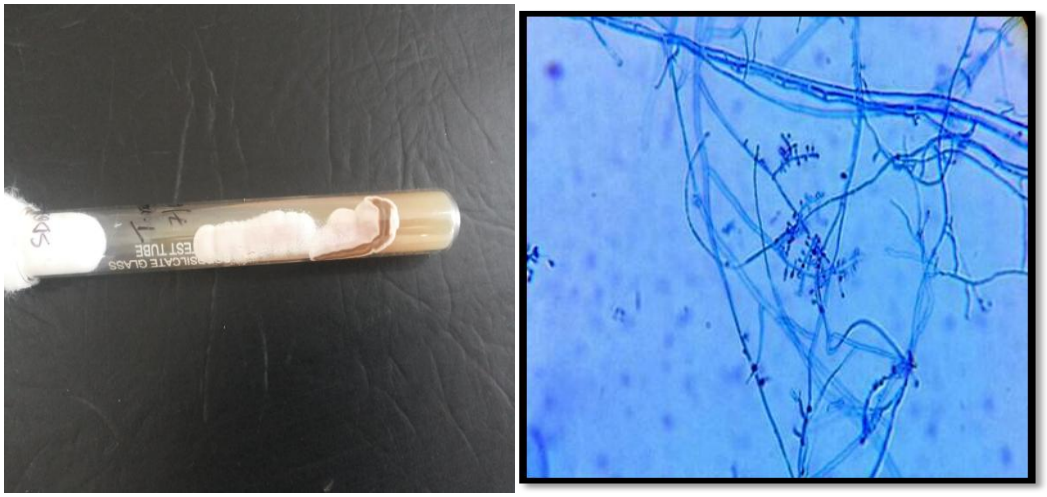


Fig.13 *Trichophyton verrucosum*

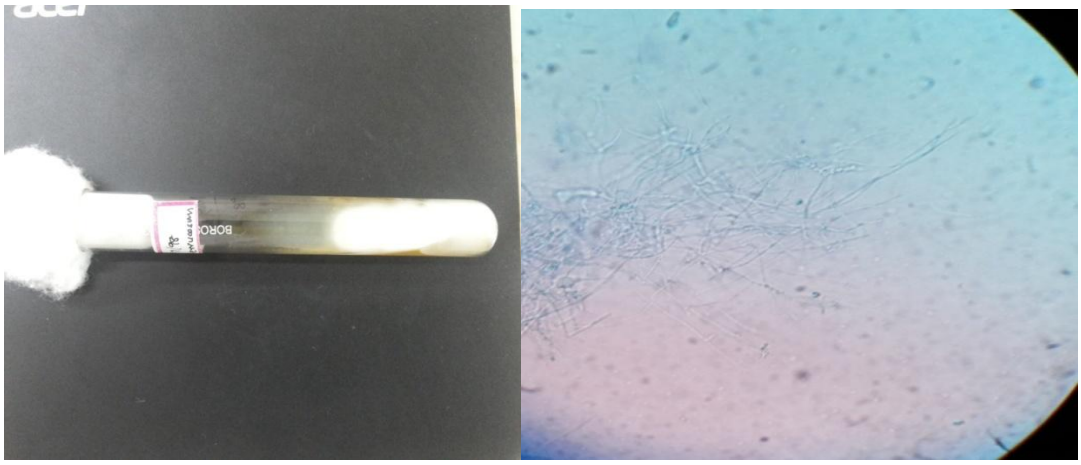


Fig.14 *Trichophyton tonsurans*

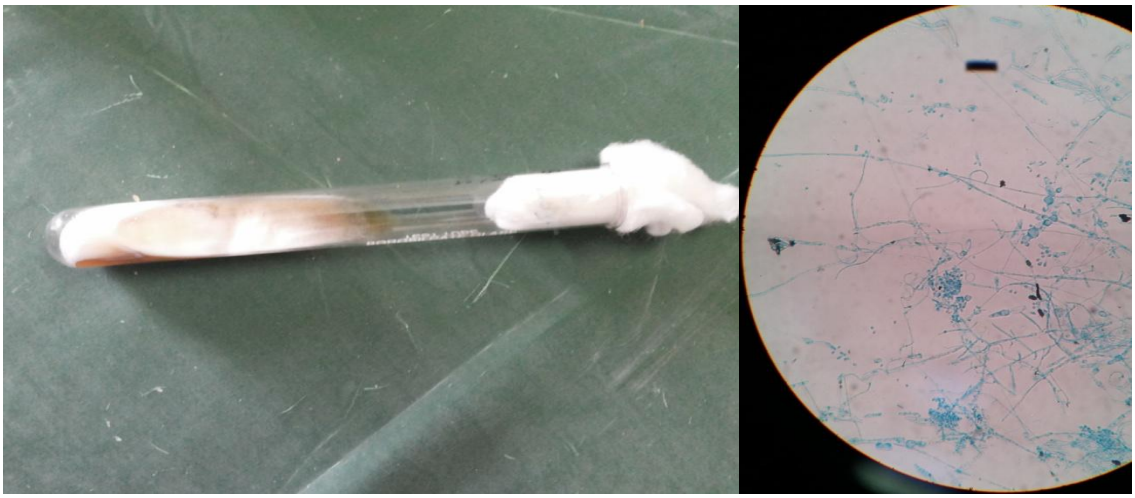


Fig.15 *Trichophyton violaceum*

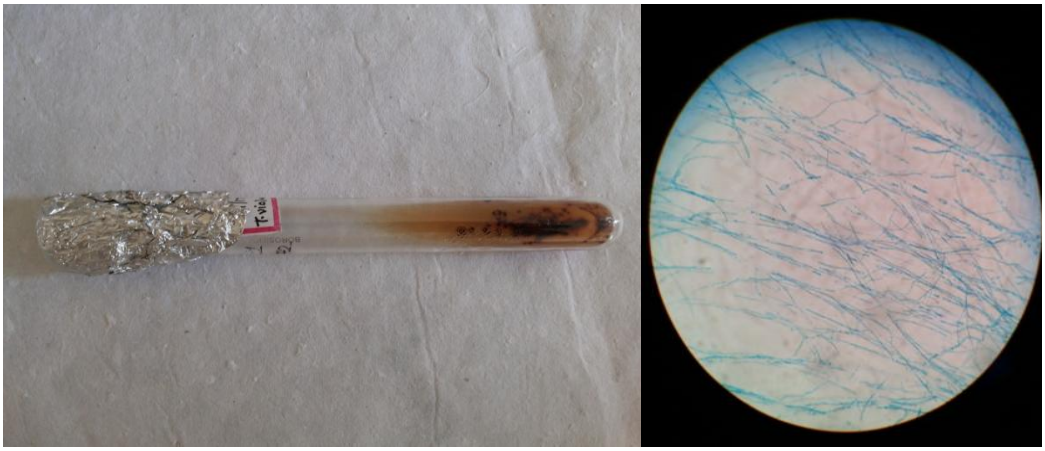


Fig.16 *Microsporum gypseum*

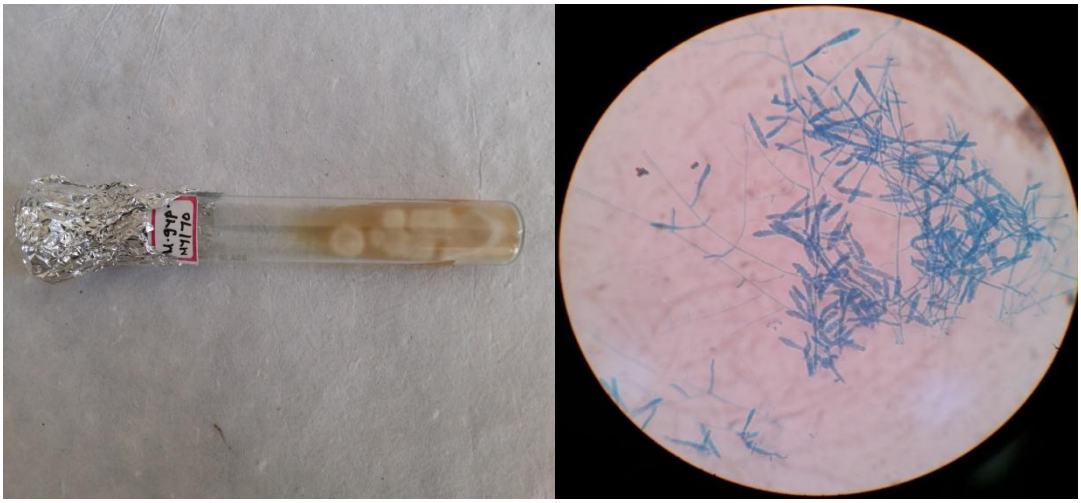


Fig.17 *Epidermophyton floccosum*

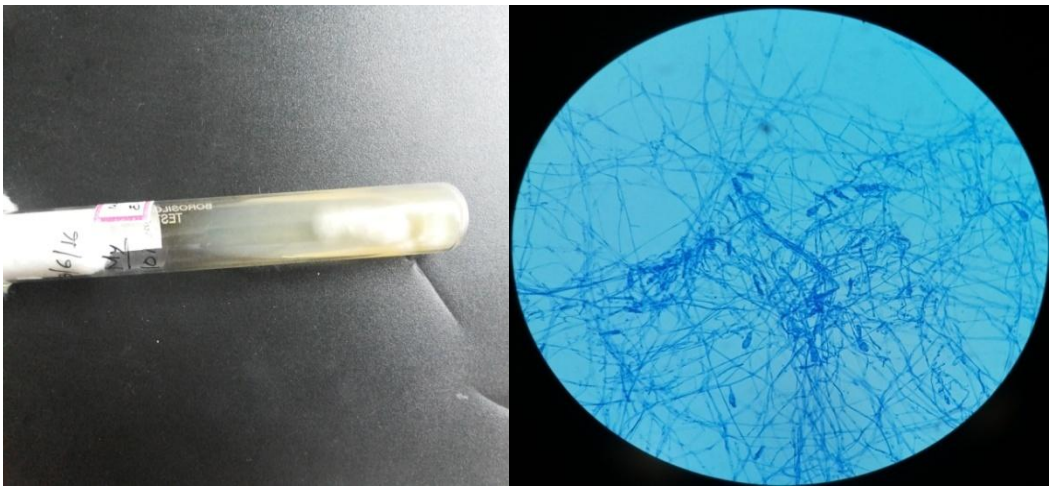
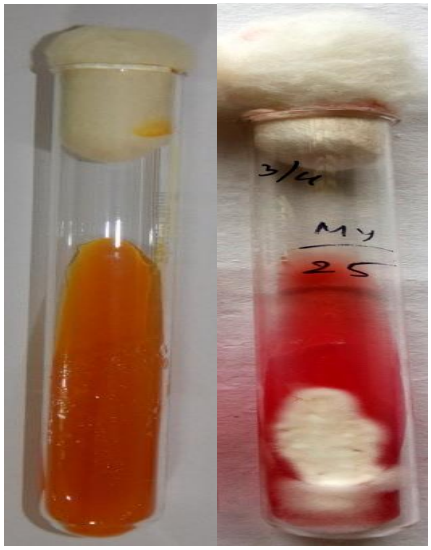


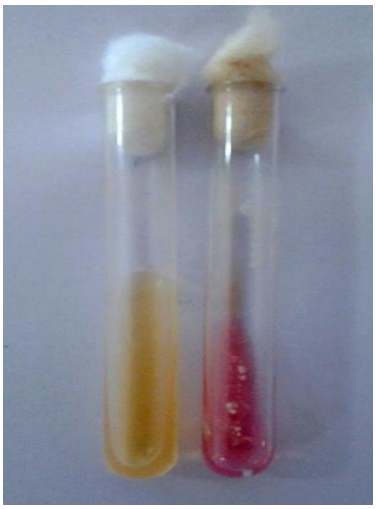
Fig.18 Dermatophyte test medium



(a) **(b)**

(a) Uninoculated medium
(b) Inoculated medium showing growth and colour change

Fig.19 Urease test - positive for *T. mentagrophytes*



Urease medium shows color change from yellow to pink

In this study, Tinea corporis was the most common clinical type 31%, followed by T. unguium 26% and T. cruris 25%. A study by Monika *et al.,* (2016) also showed T. corporis to be the most common clinical type followed by T. unguium which correlates with our study. Tinea capitis in our study was seen in 4% cases which is comparable to the study done by Sumit *et al.,* in which the number of cases was 4.4%. In our study most of the Tinea capitis cases were in pediatric age group. High occurrence of tinea capitis in less than 10 years of age may be due to lack of secretion of fungistatic sebum by scalp before puberty. In the present study, out of 100 samples collected, 69% were of skin, 27% of nail and 4% of hair. This study coincides with the study by Bhatia *et al.,* (2015) in which skin comprises of 72.77%, nail 23.26% and hair 3.96%.

Direct microscopy by KOH preparation plays an important role in diagnosis of fungal infections but culture gives definitive diagnosis. KOH positivity in our study was 51%. Various other studies revealed KOH positivity rates ranging from 46.8 % to 82%.

Culture positivity in our study was 40% which is comparable to a study by Noronha *et al.,* (2016) were also the positivity was 40%. Other studies showed rates ranging from 39% to 58%.

Our study showed a KOH and culture positivity in 36% which correlated with a study by Hanumanthappa *et al.,* (2012) (36%). KOH positivity and culture negative results were shown in 15% of cases in our study which correlated with a study by Singh *et al.,* (2016). KOH negative and culture positive results were shown in 4% cases which were close to 7% also seen in the study by Singh *et al.,* KOH and culture negative results were seen in 45% cases in our study.

In our study, 15 cases were KOH positive and culture negative which could be due to non-viability of fungal elements in these cases. Four specimens that were negative in KOH examination revealed growth of dermatophytes on culture medium. This may be because the fungus could have been in an inactive sporulating phase that is difficult to be seen by microscopy but able to grow in appropriate media.

The present study shows a sensitivity of 90% which correlates with the study by Ardakani *et al.,* (2016) which showed a sensitivity of 91.9%. The specificity was shown to be 75% which was relatively closer to the study by Krishna Santhosh *et al.,* (2015) which showed 83.46%. The negative predictive value was 91.83% in our study which was in concordance with the study by Ardakani *et al.,* (2016) which showed 98.6%. The positive predictive value was 70.58% which varied with other studies (Table 6).

In this study, the predominant species isolated was *T.tonsurans* (17%) followed equally by *T. mentagrophytes* (8%) and *T. verrucosum* (8%). The least common species were *T.violaceum* (1%) and *M.gypseum* (1%). This study correlates with a study done by Grover and Roy (2003) in North east India and by Irene Weitzman *et al.,* (1998) New York who also reported *T. tonsurans* as the commonest species. Dr.Shilpa Dayanand *et al.,* (2016) reported *T.tonsurans* as the second most common isolate after *T.mentagrophytes* in their study. Here in Gandhi hospital, Musheerabad we had a predominance of *Trichophyton tonsurans*. Most of the patients with *T. tonsurans* were housewives and students (Tables 7–9). This can be correlated due to the persistent nature of this species in indoor environments, and its ability to be transmitted through asymptomatic carriers.

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